A POTENTIAL COLLECTION METHOD FOR *AGAPETA ZOEGANA* (LEPIDOPTERA: COCHYLIDAE), A KNAPWEED-ROOT-FEEDING MOTH

Sheila M. Fitzpatrick
Agriculture Canada Research Station
6660 N.W. Marine Drive
Vancouver, B.C. V6T 1X2

ABSTRACT

This paper describes a method for collecting living, undamaged *Agapeta zoegana* (L.) moths, especially recently mated females. The objective was to gather this potential biological control agent for subsequent distribution to land infested with knapweeds (*Centaurea* spp.) Sweep-netting and baiting techniques were inappropriate collection methods, because the moths were delicate and did not appear to forage. The moths did not move to the plant tops at particular temperatures or times of day and therefore could not easily be collected by aspiration. However, males and virgin and mated females within large field cages were attracted to UV light and, during their daily period of reproductive activity from dusk to midnight, could be collected in a *Heliothis* trap (Sentry) illuminated by a blacklight. In the open, neither this method nor a mobile-blacklight technique were successful in 1988, but both warrant further work. Results are discussed in the context of *A. zoegana* establishment in B.C.

INTRODUCTION

Diffuse (*Centaurea diffusa* Lam.) and spotted (*C. maculosa* Lam.) knapweed, introduced from Europe in the early 1900's, pose a serious threat to range- and pasture-lands in B.C. (Cranston, 1980). The knapweeds outcompete native forage species on disturbed or over-grazed sites, and are of low value as forage (Harris and Myers 1984). Chemical control of knapweed in most areas is neither economically practical nor environmentally desirable (Cranston 1980). Therefore, recent research has concentrated on introducing biological control agents from knapweed habitats in Europe (e.g. Harris and Myers 1984; Muir and Harris 1986, 1987).

The knapweed-root-feeding moth, *Agapeta zoegana* (L.), was introduced from Europe in 1982, 1983 and 1984 (Muir and Harris 1987). However, unlike previous releases of other natural enemies of knapweed (the seed flies, *Urophora affinis* (Frfld.) and *U. quadrifasciata* Mg.; the moth, *Metzneria paucipunctella* (Zeller) (Harris and Myers 1984); and the beetle, *Sphenoptera jugoslavica* (Obenb.) (Powell and Harris 1986)), introduction did not result in establishment (Muir and Harris 1987). Efforts to import enough *A. zoegana* larvae for subsequent releases were unsuccessful, since many larvae shipped from Europe died from parasitism and other factors, and because knapweed habitats in Europe were fast disappearing (Muir and Harris 1987). Therefore, a propagation facility, operated by the B.C. Ministry of Forests, was set up at the Agriculture Canada Research Station in Kamloops, B.C.

Since 1985, *A. zoegana* has been reared successfully on cultivated knapweed enclosed in large steel-frame field cages, then released onto knapweed infestations in B.C. (Muir and Harris 1987). It is hoped that *A. zoegana* will become established and amenable to collection from these sites for distribution elsewhere (R. Tucker, pers. comm.). However, there is little evidence of establishment to date.

I have attempted to develop a technique for collecting large numbers of undamaged *A. zoegana* moths, especially recently mated females. I considered three methods: sweep-netting, as used for the two *Urophora* species (Harris 1986a,b) and for *S. jugoslavica*; attraction to sugary baits (Borror *et al.* 1976); and attraction to a blacklight live-trap (Frost 1952, Mikkola 1972). Experience showed that *A. zoegana* moths were too delicate to be collected by sweep-netting and unlikely to be attracted to sugary baits, as adults have never been seen nectaring, either during the day (V. Fediuk, H. Müller, pers. comm.), or at night (pers. obs.). However, *A. zoegana* moths are attracted to UV light between dusk and midnight (Tucker and Fediuk 1987). Therefore, a blacklight live-trap seemed the collection method most likely to succeed.

To determine the optimum time for trapping, I quantified nocturnal activity patterns. Because light traps often attract more males than females (Mikkola 1972), I paid particular attention to reproductive behaviour that might result in male- biased catches. I also observed diurnal activities to see if the moths ever moved up to the plant tops from which they could be collected by aspiration.

MATERIALS AND METHODS

All observations of *A. zoegana* activity were carried out from June until August, 1988, on moths maintained in 12 steel- frame field cages (3 x 3 x 2.5 m high) at the Kamloops rearing facility. Knapweed (predominantly spotted) within the enclosures was planted from seed, watered, weeded and fertilized. *A. zoegana* moths, which are bright yellow and ~1 cm long, began emerging from below-ground pupation sites in mid-June. Although moths apparently do not nectar, two feeders, each consisting of a honey-soaked wick in a 50-ml Erlenmeyer flask, were suspended 5 cm above the knapweed canopy in each cage, and renewed every few days. Mated females oviposited on knapweed foliage from June until August, and neonate larvae migrated to the roots where they fed, reducing the plant vigour, until pupation. Predators such as ants and spiders were excluded by applications of insecticide (carbaryl) around the outer boundaries of each cage. Predators seen within cages were killed by hand.

To quantify the diurnal movement of these sedentary moths, I measured their heights within the canopy as a function of time and temperature. Temperatures were read from a max-min thermometer suspended 5 cm above the tallest knapweed plants in one of the cages.

At night, moths perching within or flying above the canopy were not easily seen. Therefore, I compared day- and nighttime activity by counting the number of moths perching on the cage walls above the canopy. Night observations were carried out by the light of a flashlight dimmed with several layers of paper towel and filtered (Kodak Wratten #29) to exclude all wavelengths but red, to which moths are least sensitive (Mikkola 1972). As observations indicated that moth activity was greatest at and after dusk, I assessed reproductive activity at this time by observing females confined in net sleeve-cages (45 x 15 cm) placed over knapweed plants. The mating status and egg complement of these females was determined by dissection. *A. zoegana* males transfer a spermatophore (a mass of sperm and accessory gland secretions enclosed in a cuticular sac (Rutowski 1979; Drummond 1984) to the female reproductive tract during mating. Tracts of mated *A. zoegana* females contained either a full spermatophore or one or two partially or fully collapsed cuticular sacs. Females have two ovaries, each consisting of four ovarioles filled with oocytes (Fitzpatrick 1988), most of which were filled with yolk and yolk precursors and appeared white, while those nearest the terminal filament (Happ 1984) were smaller and clear.

The blacklight live-trap was a *Heliothis* trap (Sentry; and see Webster *et al.* 1986) suspended 15 cm above the tallest knapweed plants, and illuminated from the top by a mining-type blacklight (principal wavelength 360 nm; Fig. 1). The trap's lower cone was covered with white organdy cloth to enhance UV reflectance. Knapweed below the trap was parted to allow a white cloth to be placed there. Care was taken to shield the worker's eyes from direct UV rays. Power was provided by a portable Honda generator of 1 kW. In one instance, the blacklight was placed behind a sheet of white cotton stretched over a frame (20 x 20 cm) and mounted on the front of a four-wheel-drive all-terrain- vehicle (Honda 4-Track) to provide a moving collection device. Moths needed for field tests of collection devices were aspirated with an Insect Vac (Bioquip) from field cages.

The data were tested by analysis of variance (ANOVA) followed, if appropriate, by Tukey's test. Chi-square tests were applied to frequency data.

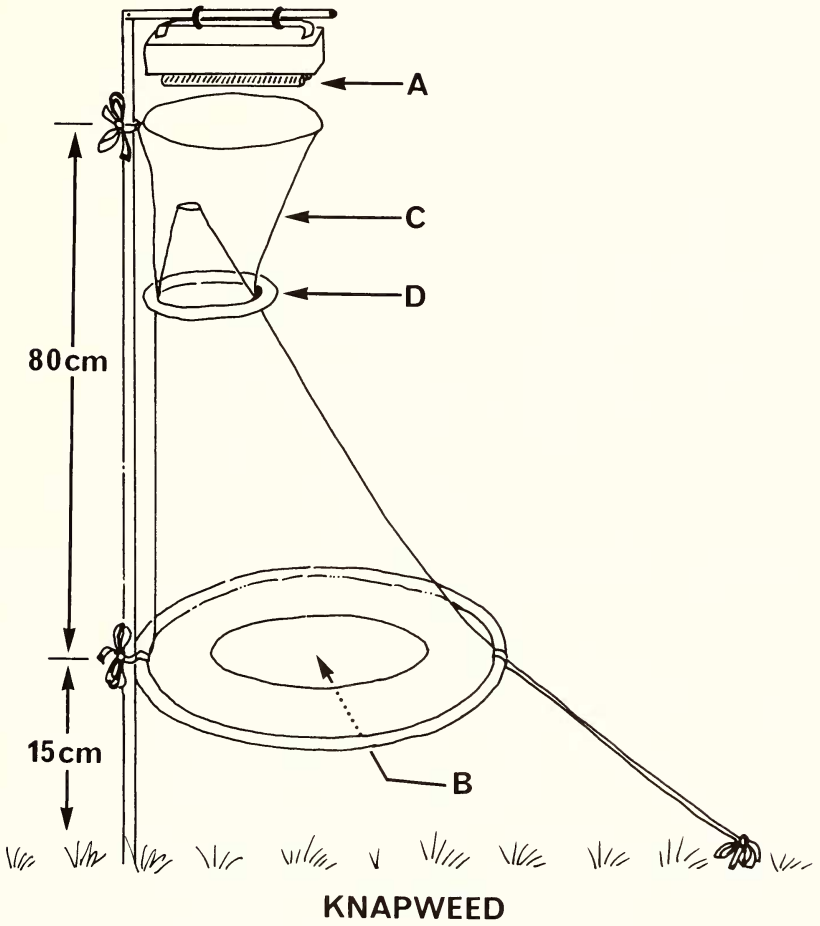

Figure 1. Schematic lateral view of blacklight live-trap. Moths, attracted by blacklight suspended at (A), enter the lower cone (B) of the *Heliothis* trap (Sentry) and fly up to the containment chamber (C), which can be removed by releasing Velcro at (D).

RESULTS AND DISCUSSION

Diurnal and nocturnal activity

A. zoegana moths remained within the knapweed canopy during the day, rarely flying. Of 28 moths observed every 2 h on June 24, 53% remained in one place from 0800 h (20.0°C) until 1500 h (30.0°C). On warm days (e.g. July 14; Fig. 2B) most moths were found in the middle to upper canopy, while on an unseasonably cool, windy day (June 30; Fig. 2A) they remained in the lower half. The moths showed no daily vertical migration to the top of the canopy, although in one case (June 30-July 1) their mean height was significantly greater at 0800 h than at 2000 h the previous evening (Fig. 2; ANOVA on heights). Therefore, aspirating the moths from plant tops was not a feasible collection method.

From morning until mid-afternoon, *A. zoegana* moths were usually difficult to disturb. They were most easily startled into flight in late afternoon and early evening, when they made short flights of 1-2 s to nearby plants or cage walls. About dusk, many of both sexes flew in 3-4 s zigzagging flights up onto the cage walls above the canopy (Fig. 3; *cf.* Muir and Harris, 1987). Despite efforts to control predators, spiders caught many of the moths perching on the walls, particularly early in the season (Fig. 3). The moths did not fly during cool, cloudy, windy weather.

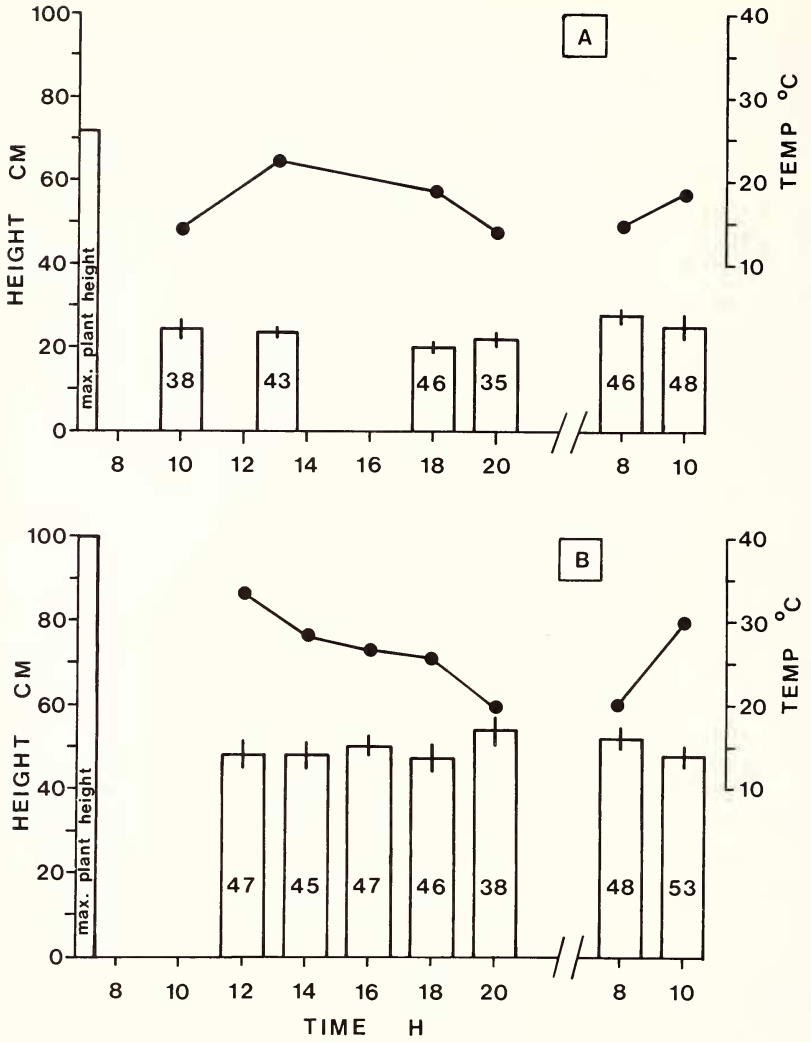

Figure 2. Mean heights (± 1 standard error) of *A. zoegana* moths perching within the knapweed canopy in field cages at the Kamloops Research Station on (A) June 30 - July 1 and (B) July 14-15, 1988. Number of moths observed is shown within each histogram. Daytime temperatures are shown ●——●. Minimum temperatures, recorded about dawn, were 10.0°C on July 1 and 5.0°C on July 15.

Female activity was monitored on three nights. On June 16, two females were placed in a sleeve cage over a spotted knapweed plant, and observed hourly from 2200-0400 h (22.0-13.5°C). Both moved to the top of the cage at dusk (~2200 h). One female was observed ovipositing at 2200, 2300 and 2400 h, while the other was seen in the "calling" posture, described by Turgeon and McNeil (1982), at 2300 and 2400 h. Both females then remained stationary at the top of the cage for the rest of the night. On June 23, six females were confined to a sleeve cage and observed every 15 min from 2100-0030 h (18.5-11.0°C). The same females were observed every 20 min from 2100-2320 h (22.5-18.5°C) on June 24. One female began ovipositing several minutes before 2100 h on both evenings, and continued in bouts until 2245 h (16.0°C) June 23 and 2240 h (18.0°C) June 24. The remaining five females moved to the top of the cage between 2145 h (17.5°C) and 2300 h (16.0°C) June 23, and between 2100 h (23.0°C) and 2140 h (21°C) June 24, where they alternately fluttered and perched. None of the five was observed calling or ovipositing, and all six stayed motionless near the top of the cage after 2300 h.

The female that called on June 16 contained 189 white eggs but no spermatophore, indicating that she had not been mated, while the other deposited ~100 eggs on the cage and contained 55 white eggs plus a partially collapsed spermatophore. Status of the six females observed on June 23-24 is shown below.

	Number of eggs			
Female	White	Clear	Total	Spermatophore
1	128	79	207	1
2	252	149	401	0
3	141	127	268	0
4	51	80	131	1
5	140	136	276	0
6	160	140	300	?

The only female seen ovipositing on those nights was #4, identified by her worn appearance. All the females dissected in the course of this study (Fitzpatrick 1988) contained more eggs than previously reported for this species (Müller *et al.* 1988).

Since moths of both sexes were active from dusk until midnight, I ran the blacklight trap during that period. I expected that the trap might capture proportionally more males, which were probably flying through and above the knapweed canopy in search of mates, than females which, although found above the canopy at dusk, probably returned to knapweed plants shortly thereafter to call or to oviposit.

Blacklight-trap tests: Within field cages

The blacklight trap (Fig. 1) was tested on four occasions. On the first, it was used from 2100 h on June 30 (1.5 h before total darkness) until early dawn at 340 h on July 1, in a field cage containing 10 males and 16 females. The minimum temperature that evening was 10.0°C. To encourage moths to fly up out of the canopy, the plants were disturbed with a stick at 2300 h (11.0°C) and 2325 h (12.0°C). At least 10 *A. zoegana* moths were observed in and on the trap at 2300 h. The next morning, five males and three females (at least one mated) were recovered from the trap. Although this sex ratio did not differ statistically from that in the cage, 13 of the 16 females were not trapped. This may have been due to windy, cloudy conditions and unseasonably cool temperatures that day and evening. *A. zoegana* females have larger body masses than males (pers. obs.), and may need a higher ambient temperature than males to initiate and sustain flight (as do *Thymelicus lineola* (Ochsenheimer) females (Pivnick and McNeil 1986)).

On July 14-15, the trap was illuminated from 2130-0300 h (15.0-8.0°C) and the plants were disturbed with a stick at 2200, 2300 and 2330 h. The trap caught 36 males and 13 females (six mated, four unmated, one of unknown status). This male-biased ratio was not significantly different from the ratio of 45 males to 19 females in the cage. All six untrapped females had been mated.

To determine if canopy disturbance had any adverse effect on trap catch, plants were left untouched during the following two tests. On July 15-16, from 2130-0300 h (13.0-7.0°C), the trap caught 40 males and five mated females. Only three males and three females were not trapped. The sex ratio of trapped moths was not different from the original male:female ratio. On July 28-29, when the trap was run from 2115-0300 h (22.0-10.0°C), 24 males and 4 mated females were collected. This ratio was not different from the original ratio in the cage. Of the six females not trapped, five were mated.

Thus the trapping method used was effective over a short range. The trap catch was neither increased nor reduced by flushing moths out of the knapweed.

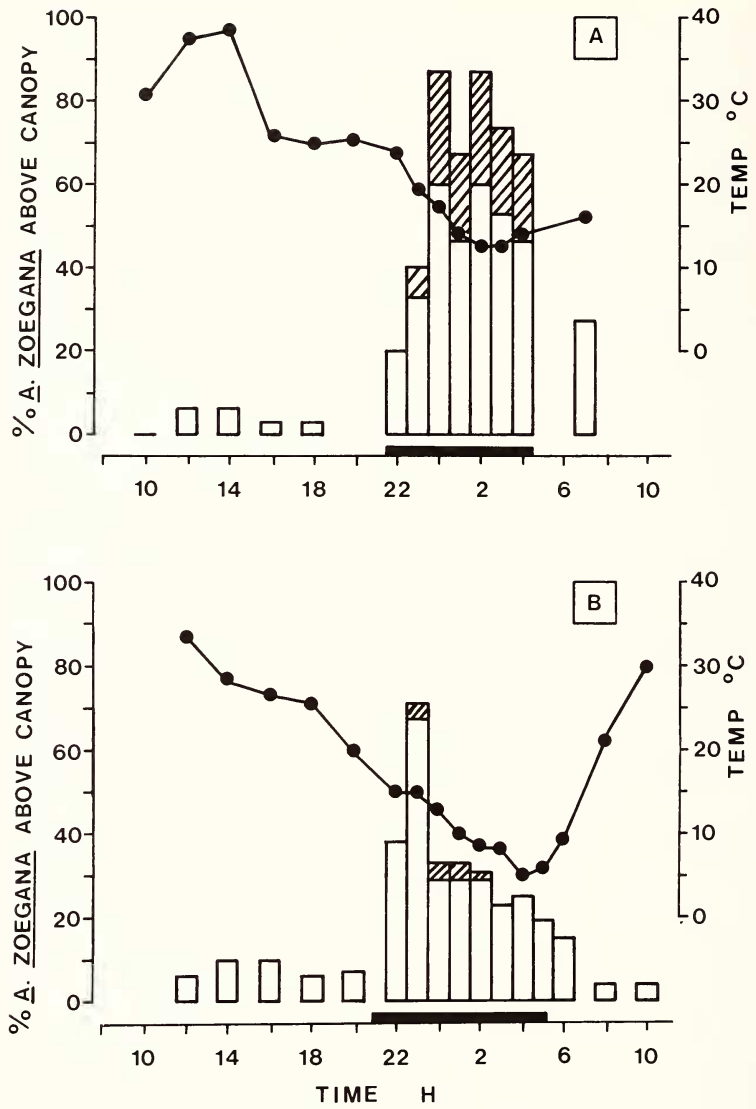

Figure 3. *Activity of A. zoegana* moths observed inside field cages on (A) June 16-17 and (B) July 14-15, 1988. Histograms show the percentage of moths seen above the knapweed canopy on cage walls. Total number of moths observed was: (A) 35 from 1000-1800 h and 15 thereafter; (B) 52 until 0600 h and 55 thereafter. Hatched portions of histograms show moths captured by spiders. Scotophase (dusk to dawn) is shown by solid bars along X-axes and temperatures are shown ●——●.

Blacklight-trap tests: In the open

The blacklight trap was tested three times in the field. The first test took place at a 1987 release site near Clearwater, where *A. zoegana* larvae had been recovered early in 1988 (V. Fediuk, pers. comm.). Two *A. zoegana* moths, one worn and one apparently newly emerged, were observed in this area at 2230 h July 21. The trap was illuminated from 2130-0300 h (19.5-12.0°C) and knapweed in a 50-m radius around it was disturbed with a stick at 2200, 2230 and 2300 h. A single *A. zoegana* male was captured, with many other insects. On July 22,

two workers spent a total of 4 h *or* 2 h each examining all knapweed plants in a 100 x 10 m area surrounding the trap. No *A. zoegana* were sighted there, nor were any seen during a wider but less-thorough search. Given the sedentary nature of the moths during the day and the fact that both searchers were accustomed to looking for *A. zoegana*, it is unlikely that the bright yellow moths went unnoticed. There were probably too few *A. zoegana* to allow for an accurate test of the trap.

An established population of *A. zoegana* could not be found, so the next two tests used moths aspirated from the field cages at the Kamloops Research Station and released at a nearby spotted knapweed infestation. At 1030 h on August 4, 100 moths (51 males and 49 females) were released onto two clumps of knapweed 20 m apart (~50 moths/clump). Moths flew to the knapweed immediately upon release. Within 1 min of release, one *A. zoegana* moth had to be rescued from an ant that was dragging it away. Although some moths probably fell prey to the numerous ants in the area, at least 10 moths were observed at the release site 10 h later. The trap was set up midway between the two release points, and run from 2100-0300 h. The knapweed was disturbed with a stick every half-hour from 2130 to 2300 h (25.0, 23.0, 21.0 and 20.0°C, respectively), but only three males were caught, and all were in the trap by 2300 h.

Thus the blacklight trap, which worked well in a small enclosure, was not effective under these field conditions. Since *A. zoegana* adults would not come to the blacklight in the field, we attempted to take the blacklight to them.

In the final test, 136 moths (53 males and 83 females) were released on three clumps of knapweed (~45 moths/clump) at 1700 h on August 18. The blacklight was attached to the front of a four-wheel all-terrain vehicle (ATV) at headlight level, and covered with a piece of white cotton, 20 x 20 cm, stretched over a wooden frame. At 2115 h (15.0°C), the ATV made 5 non-overlapping passes through the release area. Three male *A. zoegana* landed on the cloth and remained there long enough to be aspirated off.

Neither blacklight method was a success but both warrant further testing. The first test at Clearwater may have failed due to a paucity of *A. zoegana*. The second and third may have failed because the moth's behaviour was altered by capture and transport, or because released moths were quickly taken by predators. More work with established field populations of *A. zoegana* is needed.

A. zoegana establishment in B.C.

Some of these results suggest that ecological factors may account for the apparent failure of *A. zoegana* to become established, *e.g.* climatic adaptation. *A. zoegana* habitats in Europe are generally warm enough for the insect to complete two or three generations per year (Müller *et al.* 1988) whereas in the B.C. interior, *A. zoegana* is restricted to one (Muir and Harris 1987). Other factors are soil-moisture conditions and type of knapweed. Released *A. zoegana* seem most likely to survive on moist, cool, stands of spotted knapweed (*e.g.* Clearwater) rather than on dry, warm sites where diffuse knapweed predominates (V. Fediuk, R. Tucker, pers. comm.). Diffuse knapweed often germinates from seed each spring and dies in fall, making it impossible for root-inhabiting insects to overwinter (V. Fediuk, pers. comm.).

Plant vigour probably affects larval development and adult fecundity. Larval development on cultivated *vs.* field knapweed has not been assessed, but it is known that the Kamloops rearing facility yielded females containing from 150-400 eggs (Fitzpatrick 1988), fecundities greater than the maximum of 95 eggs laid by females reared in Europe from field-collected larvae (Müller *et al.* 1988). It is important to know what proportion of moths reared on cultivated knapweed will leave progeny able to survive on field knapweed.

Nothing is known of predation on *A. zoegana* adults in Europe or B.C., although several parasitoids and predators of larvae in Europe have been identified (Müller *et al.* 1988, 1989). My observations suggest that predation by ants and spiders may represent a significant mortality factor to *A. zoegana* moths here.

Finally, the sex ratio of released moths and the timing of release deserve consideration.

Early in the emergence period, males predominate (Muir and Harris 1987). Prior to 1988, the moths were not sexed before release, thus some areas may have received males only. Releases should probably coincide with the appearance of new growth because females apparently prefer to oviposit on succulent tissue (H. Müller, pers. comm.), whether on plant tops (Fitzpatrick 1988; V. Fediuk, pers. comm.) or on rosettes (Müller *et al.* 1988). Foliage quality at the oviposition site may be important to the survival of neonate larvae, which appear to mine the foliage slightly on their way to the roots (unpub. data).

Further work on any of these is needed.

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